# Association of Versican with Dermal Matrices and its Potential Role in Hair Follicle Development and Cycling

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**Versican is a member of the group of aggregating proteoglycans involved in matrix assembly and structure and in cell adhesion. We examined changes in the distribution of versican in mammalian skin, with emphasis on hair follicle development and cycling. In adult human skin, immunostaining for versican appeared predominantly in the dermis, with intense staining of the reticular dermis. Weak staining was observed at the dermoepidermal junction and the connective tissue sheath of hair follicles. Versican expression was also noted in the reticular dermis of rat skin, within dermal papillae, and possibly associated with follicle basement membranes. During mouse hair follicle development, versican was not expressed until the hair follicles were beginning to produce fibers. With follicle maturation, versican expression intensified in the dermal papillae, reaching a maximum at the height of the growth phase (anagen), after which it diminished as the end of this phase approached. Versican immunoreactivity in the papillae decreased further during catagen and was absent from these structures during telogen. However, intense staining for versican was then observed in the neck regions of telogen follicles. As the follicles entered the next hair cycle, versican disappeared from the necks and was again seen in the dermal papillae when follicles began producing fibers. This type of expression continued throughout subsequent hair cycles and is unlike any other dermal papilla component. The results of this study are consistent with a distinct supportive role for versican in the follicle matrices during hair follicle morphogenesis and cycling.** ***Key words: proteoglycan expression/human/mouse. J Invest Dermatol 105:426–431, 1995***

Versican is a member of a family of large, aggregating proteoglycans that primarily bear chondroitin sulfate glycosaminoglycan side chains [1]. Other members of the family include aggrecan [2], neurocan [3], and brevican [4]. These chondroitin sulfate proteoglycans (CSPGs) exhibit strong hyaluronan-binding capacity and form a group termed matrix hyaladherins [5]. As well as participating in matrix assembly and structure, these hyaluronan-binding proteins modulate hyaluronan function and are involved in cell-cell and cell-matrix adhesion, including cell migration. In addition to their localization to the extracellular matrix (ECM) of cartilage, they are distributed in a variety of tissues such as skin, tendon, brain, aorta, and lung (reviewed in [6–8]). The core proteins of these CSPGs range in size from 80 to 400 kDa and contain a hyaluronan-binding domain at the amino (N)-terminus and a lectin-like motif at the carboxy (C)-terminus.

Versican, originally identified in human fibroblasts [9], exhibits strong binding affinity to hyaluronan through its N-terminal domain [10]. It also contains a sequence of epidermal growth factor (EGF)–like repeats and a complement-regulatory protein-like domain in the C-terminal portion of its protein core; the putative glycosaminoglycan attachment sites are located in the middle region of the protein [1,11].

The exact function of versican remains elusive, although the molecule appears to have several roles. It may be involved in intercellular signaling through its lectin- and EGF-like sequences [11] and may also take part in cell recognition *via* its interaction with other ECM components and cell surface molecules [1]. It has been proposed that PG-M, a chicken proteoglycan related to versican, may affect cell adhesion *via* its chondroitin sulfate glycosaminoglycan chains and C-terminus [12]. Furthermore, PG-M, although existing in various tissues, appears to be developmentally regulated in tissues such as the limb bud and retina, indicating a role in cell signaling and behavior during morphogenesis [13,14].

Thus, the possibility exists that versican itself can also play an important role during development. Although several studies have reported changes in the expression of various other proteoglycans in the skin, we investigated the role of versican in developing skin. In this study, we describe spatial and temporal changes in the distribution of versican specifically during hair follicle development and the hair cycle.

## MATERIALS AND METHODS

**Construction of Fusion Proteins** All molecular biology reagents were purchased from International Biotechnologies, Inc. (New Haven, CT), Boehringer Mannheim Biochemicals (Indianapolis, IN), or Stratagene (La Jolla, CA) unless stated otherwise. All other chemicals, unless stated otherwise, were obtained from Sigma Chemical Co. (St. Louis, MO).

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Abbreviations: CSPG, chondroitin sulfate proteoglycan; LB, Luria broth.

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Full-length versican recombinant cDNA was isolated as described earlier [10] and was used as substrate in the polymerase chain reaction to amplify specific regions of versican. Primer sequences that contain *Bam*HI restriction sites toward their 5′ end were determined using the published sequence of human versican cDNA and the program OLIGO (National Bioscience, Inc., Plymouth, MN). These primers were used to amplify fragments that span versican cDNA from base pairs (bp) 417–1336, 1537–2353, and 5524–6400. Polymerase chain reaction products containing versican fragments of 911, 810, and 870 bp were separated on 1% agarose gels. Slices containing the appropriate fragment were excised and heated to 50°C for 2 min in the presence of 3 vol (w/v) of 6 M NaI and mixed with 1 ml of purification resin (Promega Corp., Madison, WI). The cDNA was eluted according to the manufacturer's instructions. The amplified products were subcloned into a unique *Bam*HI restriction site in the pBluescript-derived T-vector [15] and electroporated into electrocompetent DH5a host cells. These cells were incubated in 1 ml of Luria broth (LB) medium at 37°C for 45 min and plated on nitrocellulose membranes overlaid on LB/ampicillin plates (100 μg ampicillin/ml). After overnight incubation at 37°C, the membranes were transferred to LB/ampicillin plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 0.8 mg/100 ml plate) and isopropyl-β-D-thiogalactopyranoside (IPTG; 9.5 mg/100 ml plate) and incubated further at 37°C for several hours. White colonies were picked, and the plasmids were isolated by $LiCl_2$ precipitation. The versican cDNA fragments were excised with *Bam*HI, and the products were separated on 1% agarose gels. The appropriately sized fragments were purified as described above, subcloned into a unique *Bam*HI restriction site in pQE-12 (QIAGEN Inc., Chatsworth, CA), and electroporated into electrocompetent M15pREP4 host cells (QIAGEN Inc.). Transformed cells were incubated in 1 ml of LB medium at 37°C for 45 min and plated on LB/ampicillin/kanamycin plates (25 μg/ml kanamycin). After overnight incubation at 37°C, randomly selected colonies were grown in 1.5 ml of LB/ampicillin/kanamycin medium until 0.5 $OD_{600}$. Aliquots of each culture were stored at 4°C, and IPTG was added to the remaining cultures to a final concentration of 2 mM. The IPTG-containing cultures were incubated at 37°C for 3 h, and 0.1 ml of the suspension was washed in phosphate-buffered saline solution (PBS), boiled, and run on sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Cultures that produced a protein with a molecular weight close to the theoretic molecular weight of the versican cDNA protein product and that reacted with crude antiversican antiserum were considered positive. The corresponding uninduced cultures (previously stored at 4°C) were stored at −70°C in 15% glycerol. From these stocks, cultures were grown and fusion proteins were isolated on Ni-chelate affinity columns (QIAGEN Inc.) according to the manufacturer's recommendations. The purified fusion proteins were coupled to cyanogen-bromide–activated Sepharose (Sigma Chemical Co.).

**Versican Antibody Purification** Crude versican antiserum from rabbits was fractionated with 50% ammonium sulfate, dialyzed to PBS, preabsorbed with fetal bovine serum–Sepharose and an *Escherichia coli* acetone extract, and applied sequentially to versican fusion protein affinity columns. The eluates were further concentrated on Protein A-Sepharose (Pharmacia Biotechnology, Piscataway, NJ). Eluate fractions containing antibody were dialyzed to PBS. Bovine serum albumin (BSA) and sodium azide were added to give a final concentration of 0.5% and 0.02%, respectively, and the anti-versican antibodies were designated VC1, VC2, and VC3.

The antigen used for production of whole versican antibody (VC) was human recombinant versican, expressed by Chinese hamster ovary cells and isolated *via* anion exchange and gel filtration chromatography. The rabbit serum was preabsorbed against living and formaldehyde-fixed nontransfected Chinese hamster ovary cells, *E. coli* acetone lysate, fetal bovine serum–Sepharose, and BSA.

**Tissue Processing and Immunostaining** Frozen sections of rat and human tissue were prepared and stained as described previously [16,17] with antibodies against basement membrane CSPG (BM-CSPG) core protein [16] and versican.

Other skin samples were obtained from the mid-dorsal region of a B6C3-based strain of mice. For fetal tissue, adult mice were mated and the females were sacrificed to obtain fetuses at gestational days 13 to 18. Skin was also obtained from sacrificed mice between the ages of newborn and day 30. All skin samples were removed from freshly sacrificed animals. The tissues were then fixed in acid-alcohol fixative [18], washed with 100% ethanol, cleared with xylene, and embedded in paraffin (Tissue-Prep; Fisher Scientific, Pittsburgh, PA). Tissue sections were cut at 5 μm with a rotary microtome (American Optical Corporation, Buffalo, NY) and affixed to glass slides.

The paraffin sections were subjected to fluorescence immunostaining as described previously [16,17] using antibodies against versican. The primary antibody localization was detected with fluorescein-isothiocyanate–conjugated goat anti-rabbit IgG (Cappel, Durham, NC) as secondary antibody. Negative controls included omission of the primary antibody or the use of preimmune rabbit serum in place of the primary antibody. Some tissue sections were treated with 5% testicular hyaluronidase, *Streptomyces* hyaluronidase (50 U/ml), or chondroitinase ABC (0.1 U/ml; Seikagaku America, Inc., Rockville, MD) before blocking for nonspecific proteins. Tissue sections were viewed on a Nikon Optiphot microscope equipped with epifluoresence illumination optics and appropriate filters. Photomicrographs were taken on Ilford HP-5 film.

For peroxidase immunostaining, after clearing and rehydration, sections were treated with 10% peroxide for 5 min to inactivate endogenous peroxidases before blocking with 1% BSA. After the blocking step, the sections were rinsed in PBS then incubated for 40 min at 37°C with a biotinylated probe against hyaluronan (5 μg/ml PBS) [19]. The sections were rinsed in PBS; streptavidin-biotin-horseradish peroxidase complex (1:400 dilution; Zymed Laboratories, Inc., San Francisco, CA) was then applied for 40 min at 37°C, followed by stable diaminobenzidine substrate solution (Research Genetics, Inc., Huntsville, AL) to detect antibody binding.

**Western Blotting** Proteoglycans isolated from the conditioned medium of primary human fibroblasts (passage 7) were applied to diethylaminoethyl-Sepharose (Pharmacia Biotechnology), eluted with 1 M NaCl in 50 mM Tris buffer (pH 8), dialyzed to water, and lyophilized. The purified proteoglycans were treated with protease-free chondroitinase ABC or left untreated, loaded on a 4% to 20% precast polyacrylamide gel (Novex, San Diego, CA) (25 μg of total protein per lane), and subjected to SDS-PAGE. After electrophoresis, the material was electrotransferred onto Immobilon-P membrane (Millipore, Bedford, MA). The membrane was blocked with PBS containing 1% BSA and 0.05% Tween 20 and then incubated with anti-versican antibody or with normal rabbit IgG, followed by incubation with goat anti-rabbit IgG conjugated to horseradish peroxidase. Immunoreactivity was detected with diaminobenzidine substrate. Molecular weights were estimated using prestained SDS-PAGE standards (BIO-RAD Laboratories, Richmond, CA).

## RESULTS

**Specificity of Versican Antibodies** All four antibodies against versican used in this study recognized the core protein of human recombinant versican when expressed in primary human fibroblasts. Western blotting revealed a double polypeptide with an approximate molecular weight of 340 and 390 kDa after chondroitinase ABC treatment **(Fig 1)**. These estimates agree with previous reports on the size of the versican protein core from IMR-90 lung fibroblasts [11] and from MG-63 cells [10]. This characteristic double banding pattern may be generated by alternatively spliced forms of versican [12,20]. Although all four antibodies were used in indirect immunofluorescence microscopy, it was noted that VC2 was unable to detect versican *in vivo*, and is not described further. Presumably the epitopes in this domain were either conformationally unsuitable or were obscured by interaction with other tissue components.

**Versican Is Distributed in Rat Skin and Aorta** Antibodies VC1 and VC3 were used in indirect immunofluorescence microscopy on frozen rat tissue sections of newborn skin, aorta, and kidney. In skin sections, staining of the reticular dermis was noted along with decreased staining in the papillary dermis **(Fig 2*A*)**. Versican was also noted around hair follicles and within their dermal papillae **(Fig 2, *inset*)**, and was associated with the epithelial-papilla interface, possibly the basement membrane (BM). The distribution of versican in interfollicular skin was quite unlike that reported for BM-CSPG, which was restricted to basement membranes [17] **(Fig 2*B*)**. However, a similar distribution of versican and BM-CSPG was noted in the dermal papillae of anagen hair follicles. A detailed description of versican distribution in follicular development and cycling is given below.

As positive controls, sections of rat aorta and kidney were stained for versican. The proteoglycan was abundantly present in the media and, to a lesser extent, in the adventitia of the aorta **(Fig 2*C*)**, but was restricted to the ECM of major blood vessels of the kidney. No staining was associated with glomeruli (results not shown).

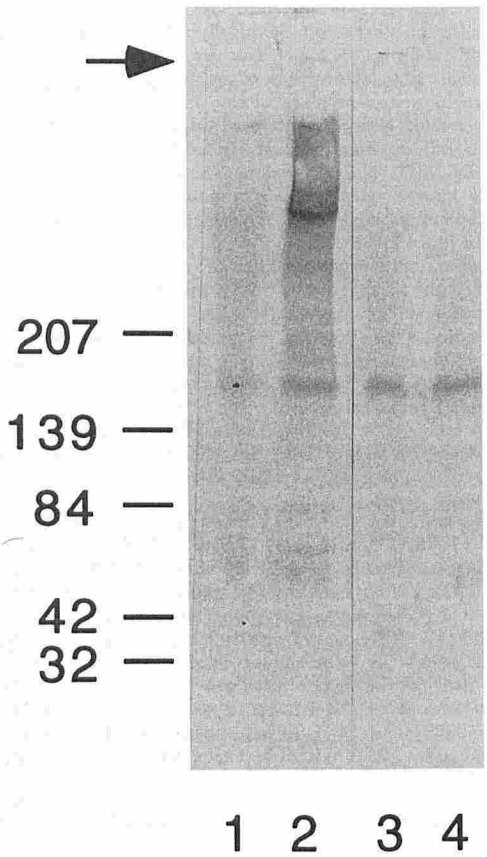


**Figure 1. Specificity of versican antibodies shown by Western blotting.** Proteoglycans from the conditioned medium of primary human fibroblasts were treated with protease-free chondroitinase ABC or left untreated. The proteoglycans were separated on a 4% to 20% gradient polyacrylamide gel (25 μg of total protein per lane) and subjected to SDS-PAGE before transfer to Immobilon-P membrane. The membrane was probed with anti-versican antibody or with normal rabbit IgG. *Lanes 1* and *3*, untreated proteoglycans; *lanes 2* and *4*, chondroitinase-ABC–treated proteoglycan. Anti-versican antibodies recognize a diffusely staining high–molecular-weight band in the untreated proteoglycans (*lane 1*) and high–molecular-weight discrete versican protein core bands of approximately 340 and 390 kDa in the chondroitinase-treated sample (*lane 2*). Proteoglycans treated with normal rabbit IgG show nonspecific staining at 175 kDa (*lanes 3* and *4*). Similar background staining is seen in *lanes 1* and *2*. Molecular weight markers (kDa) are indicated (*left*). *Arrow*, bottom of the gel loading wells.

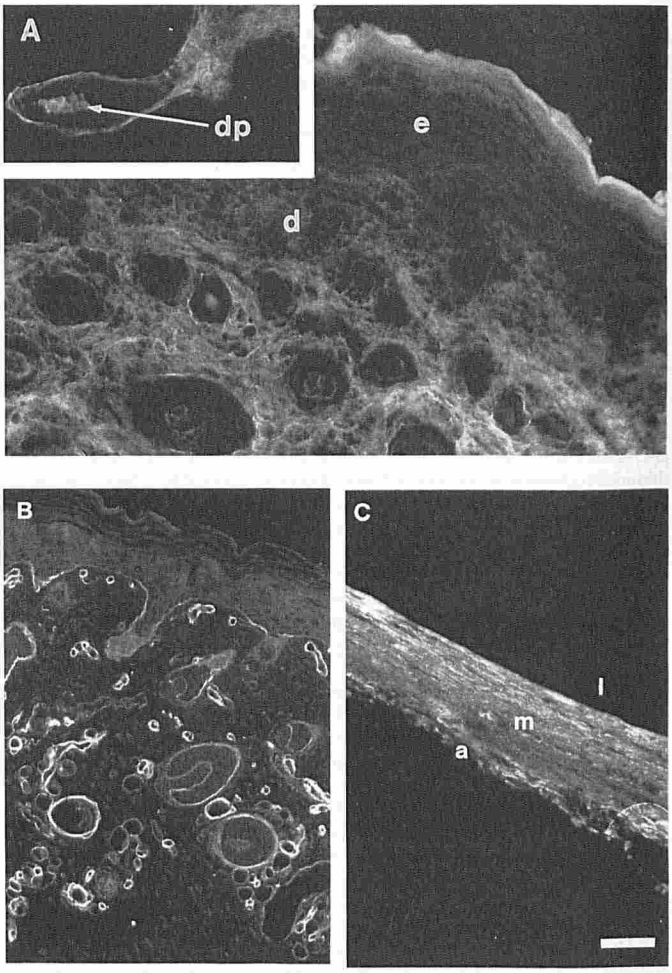


**Figure 2. Versican is present in rat skin and aorta.** Versican immunoreactivity was observed in the reticular dermis of rat skin, with decreased staining in the papillary dermis (*A*). Versican was noted around hair follicles and within dermal papillae (*inset*). In interfollicular skin, the distribution of versican was quite unlike that of BM-CSPG, which was restricted to to basement membranes (*B*). An abundance of versican was observed in the media and adventitia of rat aorta (*C*). *Bar*, 50 μm. a, adventitia; d, dermis; dp, dermal papilla; e, epidermis; l, lumen; m, media.

**Versican Is Present in the Skin and Blood Vessels of Adult Human Skin** Both VC1 and VC3 antibodies stained human skin sections and revealed large amounts of dermal versican, with more intense staining again in the reticular dermis **(Fig 3*A*)**. Weak staining was also detected in the dermoepidermal junction of scalp skin and connective tissue sheath of hair follicles **(Fig 3*B*)**. Versican was present in the pericellular matrix of sweat glands and ducts **(Fig 3*C*)**, perhaps associated with their basement membranes. Strong staining was also observed in perivascular connective tissue of large vessels and associated with elastic fibers **(Fig 3*D*)**.

**Versican Is Expressed in the Dermal Papillae During Hair Follicle Development in the Mouse** Development of the pelage or coat hair follicles begins in mouse skin at approximately 14 d of gestation. No immunostaining for versican was observed in initiating or developing hair follicles until gestational age 18 d **(Fig 4*A*)**. Equivalent control sections did not show any staining **(Fig 4*B*)**. At 18 d gestation, just before birth, the skin displays a range of follicles at various stages of development, from the condensation stage through the plug stage to nearly mature follicles just beginning to produce hair fibers; these latter follicles are those first initiated in the skin. Only these advanced follicles displayed light staining in their dermal papillae. No immunoreactivity was observed in the dermal components of less developed follicles, and none was observed in the epidermal components of the follicles or in the epidermis or basement membranes. Staining in the upper keratinized layers of the epidermis was nonspecific. Very faint, diffuse staining was observed throughout the upper dermis and in the perivascular connective tissue. As the follicles matured, staining for versican in the dermal papillae became more intense **(Fig 4*C,D*)**, reaching maximum intensity at the height of the growth phase, or anagen IV (results not shown), but it began to diminish as the follicles approached the end of this phase. The faint diffuse upper staining of the dermis persisted throughout this period.

Equivalent tissue sections pretreated with testicular hyaluronidase displayed either very weak or no staining for versican, indicative of release or destruction of the protein epitopes on versican recognized by the antibody. Versican staining was unaffected by pretreatment of sections with *Streptomyces* hyaluronidase or chondroitinase ABC (results not shown), showing that removal of hyaluronan or chondroitin/dermatan sulfate chains was not responsible for the effects noted with testicular hyaluronidase for the loss of versican protein.

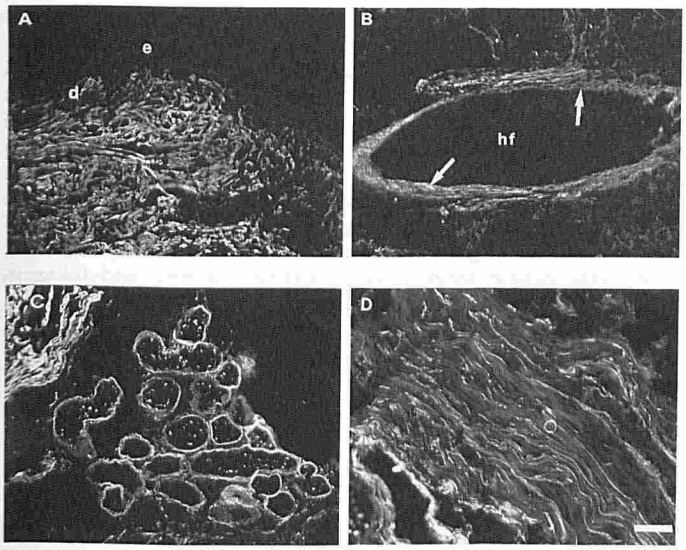


**Figure 3. Versican is distributed in the skin and blood vessels of adult human tissue.** Large amounts of dermal staining for versican were detected in human skin, with more intense staining found in the reticular dermis (*A*). Staining was observed in the connective tissue sheath of hair follicles (*arrows; B*). Versican was present in the pericellular matrix of sweat glands and ducts (*C*), with strong staining detected in perivascular connective tissue of large vessels and associated with elastic fibers (*D*). *Bar*, 50 μm. d, dermis; e, epidermis; hf, hair follicle.

**Versican Is Expressed in Dermal Papillae and Follicle Necks During the Mouse Hair Cycle** After the growth period, hair follicles enter a short transition stage, catagen, then telogen, which is the resting phase of the hair cycle. Staining for versican in the dermal papillae diminished as the follicles entered the final stages of anagen. It is interesting that not only did staining intensity for versican lessen, but it also began to retreat down the papillae until only the bases of the papillae showed staining **(Fig 5*A*)**. During catagen, the hair follicle undergoes structural changes whereby the epithelial cells surrounding the dermal papilla retreat toward the surface of the skin until, in telogen, the dermal papilla remains as a clump of cells attached to the base of the retreating epithelial column. All staining for versican disappeared from the dermal cells at this stage **(Fig 5*B*)**. However, intense versican immunostaining was observed in the "neck" regions of hair follicles in telogen **(Fig 5*B*)**.

Versican staining disappeared from the neck regions of the follicles as the follicles reformed and entered the early stages of anagen of the second hair cycle **(Fig 5*C*)**. The follicles did not display any versican immunoreactivity until they were again mature and beginning to produce fibers approximately in anagen II, when the dermal papillae were again positive (compare **Fig 5*C*, 5*D***). Staining for versican intensified in the papillae during the most vigorous growth stage **(Fig 5*E*)**, but, as in the developing follicles, it decreased as the follicles approached the end of their growth phase and disappeared as the follicles entered the catagen and telogen phases of the hair cycle.

## DISCUSSION

This report shows versican to be a widespread ECM component of rodent and human skin, with a unique pattern of distribution in murine hair follicle morphogenesis and cycling. It is most abundant in dermal matrix, particularly in human reticular dermis, and in the matrix of follicular dermal papillae. In human but not rodent skin, we also detected weak staining of the dermoepidermal junction and strong staining associated with elastic fibers, consistent with a previous report [20]. Versican is synthesized by cultured human keratinocytes, which indicates that they may then be a source of versican in this basement membrane. We did not, however,

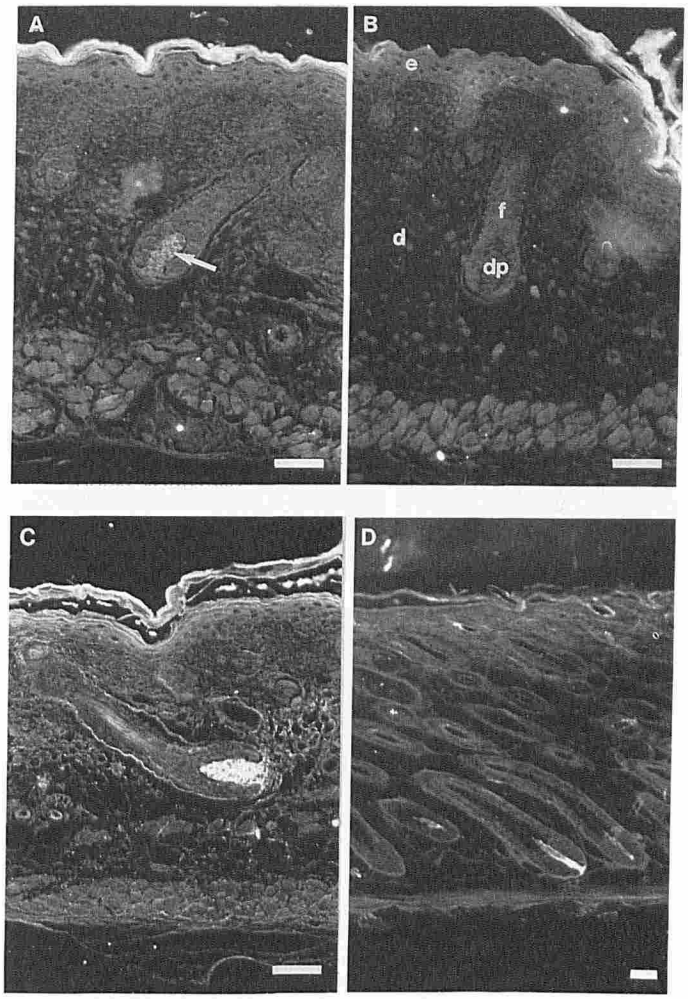


**Figure 4. Versican is expressed in the dermal papillae of developing mouse hair follicles.** Immunostaining for versican was not observed in the skin until gestational day 18, when light staining appeared in the dermal papillae (*arrow*) of follicles producing hair fibers (*A*). Control sections showed no staining (*B*). In newborn skin, versican immunoreactivity intensified in the dermal papillae of mature follicles (*C*), reaching maximum intensity at the height of the growth phase about 9 d after birth (*D*). *Bars*, 50 μm. d, dermis; dp, dermal papilla; e, epidermis; f, fiber.

observe staining of the basal keratinocytes as reported previously [20]. This may be due to differences in the antibodies used in the two studies, as different regions of the versican molecule were used in each case. The polyclonal antibody used here was prepared against whole versican (Vo form) core protein, and reactivity was noted with at least three distinct regions. These included a region approximate to the hyaluronan-binding domain, indicating that all forms of versican (PG-M) are recognized, regardless of the alternate splicing recently reported [21]. Overall, we noted considerably less intense staining of rodent dermis than of human dermis, with the exception of hair follicles and perivascular tissue. Decreased sensitivity of the antibodies, raised against human recombinant versican, to rodent tissue is not a likely explanation of our results, given the intense staining of selected tissues.

Hair follicle morphogenesis and subsequent development are complex series of events that involve extensive cell and matrix remodeling, and molecules such as proteoglycans and growth factors have been implicated in these processes [17,22]. Given the changes in the distribution of other matrix molecules, including hyaluronan, in these processes, we have carefully examined versican expression in murine skin development.

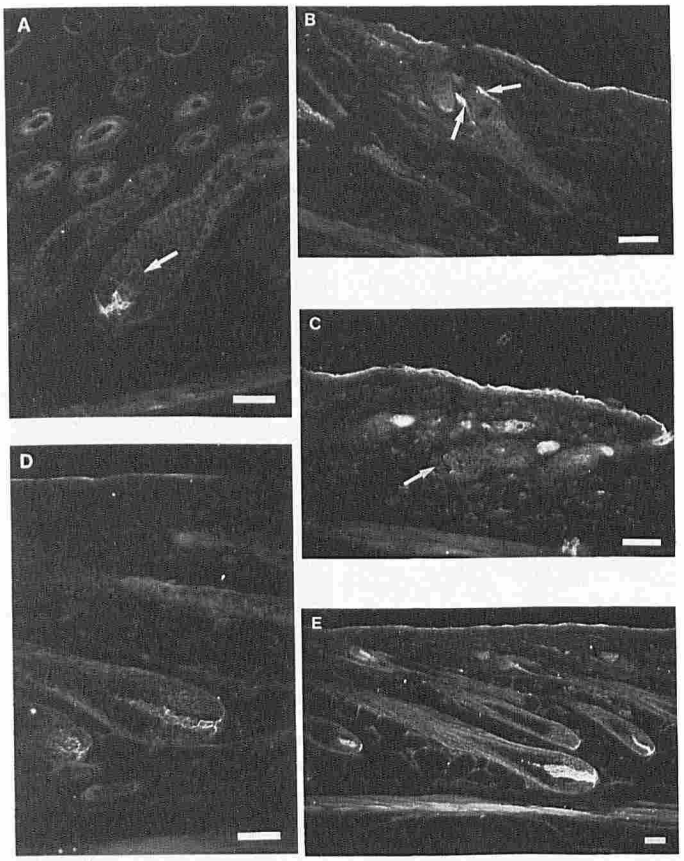


**Figure 5. Versican is expressed in dermal papillae and follicle necks during the mouse hair cycle.** As the hair follicles entered catagen (day 16), versican immunostaining retreated down the dermal papillae (*arrow*) until only the bases were stained (*A*). Versican disappeared from the dermal components of the follicles during telogen (day 19), only to appear intensely in the neck or bulge regions (*arrows; B*). As the follicles began to reform in the early stages of the next hair cycle (day 24), versican staining disappeared from the neck regions (*C*), remained absent from the dermal component of the follicle (*arrow*), and reappeared in the dermal papillae only when the follicles began again to produce hair fibers (*D*). Staining for versican intensified in the papillae during the most vigorous growth stage (*E*), but again decreased as the follicles neared the end of this hair cycle. *Bars*, 50 μm.

Hair follicle morphogenesis in the mouse begins relatively late in development. The hair coat, or pelage, follicles of the mouse begin to grow at approximately 14 d of gestation and continue initiating up to a few days after birth [23,24]. Versican was not expressed during the early stages of follicle development, first appearing in the dermal papillae as the follicles were maturing and beginning to produce fibers. It has been shown previously that the dermal papilla is essential for fiber production to occur [25]. Staining for versican in the dermal papillae became more intense, reaching maximum intensity at the height of fiber production, but began to diminish as the follicles approached the end of anagen.

Changes in expression of other proteoglycans have been noted previously during skin and follicle development and during the hair cycle [17,26,27]. BM-CSPG has a distribution through hair development and cycling quite different from that seen here for versican [17]. Both CSPG and dermatan sulfate proteoglycan (DSPG) species exhibited age-related changes in developing rat skin [26]. In fetal skin, the amount of DSPG was found to be much higher (about 14 times) than that of CSPG but, in the few days before birth, it declined at about half the rate of CSPG. The study suggested significant changes in proteoglycan synthesis and/or degradation, with greater changes shown in CSPG turnover than in DSPG. Furthermore, a greater decrease in the relative proportions of sulfated glycosaminoglycan chains (chondroitin 4-sulfate versus chondroitin 6-sulfate) was noted in CSPGs compared with DSPGs in fetal and newborn skin. Although this study did not identify specific core proteins, these results suggest that proteoglycan expression varies dramatically during the transition from fetal to adult tissue.

After anagen of the development stage, the follicles undergo cycling activity that continues throughout the life of the animal. At maturation, the pelage follicles undergo cycles of activity and rest, each cycle having three phases: anagen, catagen, and telogen [23,28,29]. As during follicle development, fiber production occurs during the anagen phase. At this most active growth stage, the follicles rapidly produce fiber and the skin is at its maximum thickness.

Versican expression diminished at the end of anagen as the follicles began to enter catagen. It is interesting that versican immunostaining decreased by retreating down the papillae until in telogen, the resting phase, all staining for versican disappeared from the dermal cells. This pattern has not been recorded for any other papilla component. Versican may then act as a specific marker of matrix changes in the unique papilla ECM. As staining disappeared during catagen and telogen, intense versican immunostaining was then observed in the neck regions of hair follicles in telogen. This region of the follicle is apical to the insertion point of the arrector pili muscle but distal to the sebaceous gland ducts, and is in the bulge area wherein resides a putative stem cell population reputedly responsible for control of the hair cycle [30,31]. CSPG in this area has been observed previously using a carbohydrate-specific antibody [32]. Because this antibody recognizes chondroitin sulfate, it is possible that it detected versican. The function of versican in this area and at this particular phase of the hair cycle is unknown, but it may be involved in providing a suitable matrix for maintenance of the stem cells before their activation at the beginning of the hair cycle.

Versican staining disappeared from the neck regions of the follicles as they entered the early stages of anagen of the next hair cycle. No versican immunoreactivity was detectable until the follicles were beginning to produce fibers in about mid-anagen, when the dermal papillae were again positive. Again, this is unlike any other dermal papilla matrix component. Staining for versican intensified in the papillae during the most vigorous growth stage, but, as in the developing follicles, it decreased as the follicles approached the end of their growth phase and disappeared as the follicles entered the catagen and telogen phases of the next hair cycle.

Expression of versican thus appears to correlate not with the proliferative activity of follicular epithelium, but rather with follicle differentiation. The epidermally derived cells surrounding the dermal papilla in the follicle bulb have a number of fates, differentiating to form the inner root sheath and the hair fiber itself. Previous studies have shown that the dermal papilla of rodents produces a unique ECM containing basement membrane components such as BM-CSPG, perlecan, type IV collagen, and laminin, but, unlike the ECM of the dermis, it lacks most interstitial collagen [33,34]. However, if papilla cells are grown under culture conditions, their matrix reverts in time, becoming similar to the matrix produced by dermal fibroblasts, and the cells lose their ability to induce hair growth [35]. Because dermal papilla cells do not constitute a population of rapidly dividing cells, even during the most active follicular growth stage of anagen, it is not likely that the intense staining for versican in the papillae is related to cellular proliferation. More likely, the molecule is part of the papilla ECM, providing a unique support system for these cells during the inductive processes of hair follicle development and cycling.

Further evidence for a supportive role is provided by the presence of versican in the bulge region of the follicles during telogen, where it may help to maintain the viability of the stem cell population. In addition, a comparison of previous data [36] and this report indicates that the expression of versican in developing and cycling hair follicles is closely correlated with that of hyaluronan.

Hyaluronan is thought to maintain the extracellular space between cells; its loss permits cellular condensation to occur [36,37]. This is illustrated during hair follicle morphogenesis as hyaluronan is conspicuously absent from the developing follicle until it reaches maturity; hyaluronan is then expressed in the matrix of the dermal papilla [36]. An inverse correlation between the expression of hyaluronan and one of its receptors, CD44, has also been shown in the developing hair follicle [36]. It is therefore possible that hyaluronan and versican interact in dermal papilla matrix, supporting the notion that hyaluronan is not preferentially involved in interactions with the CD44 class of receptors. Versican has been shown previously to bind hyaluronan through its N-terminal globular region and to modulate hyaluronan function [5,10]. It has been suggested that versican acts as a bridge between hyaluronan and cell surfaces, perhaps holding hyaluronan in place [10]. This role is supported by findings that the versican-related proteoglycan PG-M is involved in the binding of hyaluronan to both fibronectin and type I collagen [38]. In the present study, versican staining was reduced or eliminated by testicular hyaluronidase treatment, revealing a loss of protein epitopes on the core protein. However, staining was not reduced by treatment with the hyaluronan-specific enzyme, *Streptomyces* hyaluronidase, or by chondroitinase ABC. Versican is substituted with chondroitin sulfate chains, whose presence is, therefore, not solely responsible for stable interactions in connective tissue. Similarly, though versican interacts with hyaluronan, depletion of this glycosaminoglycan does not lead to a detectable loss of versican from tissue sections. Versican may undergo other important protein-protein interactions in the matrix, which are disrupted by the proteases present in commercial testicular hyaluronidase.

Although specific matrix molecules, besides hyaluronan, to which versican binds have not yet been identified *in vivo*, it can be envisioned that versican has a distinct supportive role during hair follicle morphogenesis and cycling by forming an integral part of the matrix scaffold.

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